FORMULATION AND EVALUATION OF NANOSTRUCTURED LIPID CARRIER OF TIRZEPATIDE BY USING TWEEN 80 AND POLOXAMER 188 FOR OBESITY

Abstract

The current study focuses on the formulation and the evaluation of **Nanostructured Lipid Carriers (NLCs)** for the delivery of **Tirzepatide**, a dual incretin receptor agonist indicated for the treatment of type 2 diabetes(non-insulin dependent diabetes mellitus) and obesity. Due to its large molecular weight, peptide nature, and poor water solubility, Tirzepatide faces challenges such as less oral bioavailability, rapid degradation, and limited patient conformity when the administered through conventional routes.Tirzepatide was encapsulated in NLCs using the hot homogenization followed by ultrasonication method, employing biocompatible lipids and surfactants.

Five formulations (TRZ-NLC1 to TRZ-NLC5) were developed and the characterized for size of particles, polydispersity ratio (PDI), drug entrapment efficiency, zeta potential and drug loading. The formulations exhibited particle sizes below 150 nm, PDI values below 0.25, and zeta potential ranging from -28.5 to -32.9 mV, indicating good stability and homogeneity. High entrapment efficiencies (89.8– 93.6%) and drug loading (2.95– 4.20%) were achieved. Morphological assessments via SEM, TEM, and AFM confirmed spherical and smooth-surfaced nanoparticles.

In the vitro drug release studies revealed as sustained release profile, particularly in TRZ-NLC5, supporting the potential for controlled drug delivery. Stability studies demonstrated consistent particle size and drug content over time with minimal physical changes. Overall, the TRZ-NLC formulations successfully enhanced the physicochemical stability and release characteristics of Tirzepatide.. The optimized

TRZ-NLCs may offer improved bioavailability, reduced dosing frequency, and enhanced patient compliance. Further in vivo studies and clinical translation are warranted to confirm the healing properties of this nanocarrier-based approach.

Keywords: Tirzepatide, Nanostructured Lipid Carriers, Controlled Drug Delivery, Peptide Therapeutics, Bioavailability.

Introduction

□ **NLC**

An improved method of drug delivery, particularly for these are not highly water-soluble, and it is use of nano lipid carriers (NLCs). Designed as an improved lipid nanoparticle system for the second generation, NLCs include both solid and liquid lipids stabilized by surfactants. In comparison to their forerunners, solid lipid nanoparticles (SLNs), this novel composition increases the formulation's physical stability, reduces drug expulsion during storage,and improves the drug loading capacity. Enhance bioavailability, controlled drug release, targeted distribution, and enhanced pharmacokinetic profiles are just a few of the benefits that NLCs provide, because to their nanometric size (usually below 200 nm). They are well-suited for oral, topical, parenteral, and pulmonary administration, and they excel in delivering medications across biological barriers like the skin and the blood-brain barrier. Natural lipid coatings (NLCs) have several potential uses in the pharmaceutical, cosmetic, and nutraceutical industries due to their biocompatibility, capacity to integrate hydrophilic and lipophilic medicines, and prospect for large-scale manufacturing. Their promise as a treatment is growing as new formulation procedures and methods of characterisation are developed.(Beloqui et al., 2016)

- **Background and Rationale:**

Low bioavailability , Poor water solubility and lack of site-specific administration are common problems with conventional dose forms such as injections, pills, and capsules. Negative side effects or systemic harmful may result from these limitations. Carriers based on nanotechnology, such as NLCs, have been created to combat this.(Loo et al., 2013)

Natural lipid carriers (NLCs) offer a lipid-based platform that is compatible with the absorption paths of many different medicines, including hydrophobic, hydrophilic, and

amphiphilic compounds. Additionally, NLCs are becoming more acceptable for drug delivery via oral, parenteral, cutaneous, and pulmonary routes due to their lipid components are GRAS. Particularly useful for delicate biomolecules such as peptides or nucleic acids, they shield medications against chemical and enzymatic destruction.(Naseri et al., 2015)

□ . Composition of NLC

There are mainly three parts to NLCs:

a. Solid Lipids

These give the carrier its structural integrity and serve as its backbone. Some such examples are:

1. Stearic acid monoglyceryl
2. Aspartate of glyceryl (Compritol®)
3. Acid stearicum
4. Coconut oil

Controlled release and medication retention are achieved by these lipids, which remain solid at room temperature and body temperature as well.

b. Oils and Other Liquid Lipids

Drug loading and decrease the likelihood of drug expulsion during storage, these oils

cause cracks to form in the solid lipids' crystalline structure. Here are several examples:

1. Olive acid

2.Oil containing medium-chain triglycerides

3. Propylene glycol

4. A product of Capryol®

c. Solubilizers

Emulsion stabilization and prevention of aggregation and sedimentation are achieved by these. They may exhibit ionic, non-ionic, or amphiphilic properties:

1. Polysorbate 80, or Tween 80,

2. Anhydropoloxamer 188

3. Soybean gelatin

4. Chloride salt

Particle stability, surface charge, and surfactant concentration are affected by the surfactant choice and concentration.(Naseri et al., 2015)

- **Mechanism of Drug Encapsulation**

Incorporating liquid lipids into the NLC matrix formation of a structure that is somewhat disordered. The "imperfection" in question generates gaps inside the matrix that are conducive to the accommodation of drugs. In the medicine may either be adsorbed on the surface or it may be solubilized in the lipid phase. Typically, medications that are lipophilic will embed themselves in the core, whereas compounds that are amphiphilic may reside near the interface.NLCs can release drugs in a variety of ways, including the following:The process by which the drug gently diffuses out of the lipid matrix is referred to as diffusion-controlled.Degradation of the lipids in vivo through metabolic processes is controlled by erosion.When it comes to biodegradable lipid systems, the combined mechanism is frequently observed.The composition of the lipids, the kind of surfactant, the compatibility of the medication with the lipids, and the size of particles are all factors that influence release.

- **Type of NLC**

Based on their internal morphology, NLCs classified into three primary structural models:

a. Crystal Type Imperfections

An uneven matrix with "imperfections" or voids is the result of combining different lipids. Over time, these holes lessen drug ejection and permit more drug loading.

b. Type Amorphous

makes use of lipids that are amorphous or difficult to recrystallize. This improves physical stability and inhibits the creation of organized structures.

c. The solid lipid phase contains microscopic oil nanocompartments of the Multiple Oil-in-Fat-in-Water (O/F/W) type. These sections serve as extra drug solubilization

reservoirs, particularly for highly lipophilic substances.(Ghasemiyeh & Mohammadi-Samani, 2018)

□ . Advantages of NLC

- Increased drug loading: NLCs can load more medication than SLNs, which is a major advantage. The lipid matrix's poor crystalline structure is caused by combining solid and liquid lipids. These defects allow more medication molecules to fit in the lipid core's vacancies. Drugs with low water or high lipid solubility benefit from this.

- Drug Expulsion Prevention During Storage: The highly structured crystalline structure of SLNs often expels drug molecules during storage as the lipids recrystallize into more stable polymorphism forms. In contrast, the partial disorder in the NLC matrix precludes full crystallization, preserving formulation integrity and reducing drug leakage.

- Maintaining Drug Release NLCs can adjust medication release based on lipids and surfactants. Solid and liquid lipids inhibit drug diffusion from the matrix, prolonging drug release. This characteristic reduces dose frequency and boost patient compliance, for the chronic conditions.

- Better Bioavailability: NLCs increase oral medication bioavailability in numerous ways:

a. Improved gastric solubility.

b. Anti enzymatic protection.

c. Improved lymphatic transport,

d. bypassing hepatic first-pass metabolism.

They also boost intestinal epithelial cell adhesion and absorption due to their nanosize.

- Biocompatibility/Biodegradability: NLC lipids and surfactants are usually GRAS by regulatory bodies. Biodegradable and converted into non-toxic metabolites, they reduce cytotoxicity. This renders NLCs safe for oral, cutaneous, pulmonary, and intravenous administration.

- Labile Drug Protection:Heat, light, and enzymatic activity degrade bioactive substances like peptides, proteins, and nucleic acids. The lipid matrix of NLCs protects the medication from environmental degradation, extending its shelf life and in vivo stability. (Araújo et al., 2010)

- Crossing Biobarriers: The nanoscale and lipophilic characteristics of NLCs allows them to overcome biological barriers like:

a. Transdermal medication administration stratum corneum.

b. The BBB for CNS medication delivery.

c. Intestinal mucosa improves oral absorption.

(Souto et al., 2020)

This opens up NLCs for diseases that need tailored delivery to hard-to-reach locations.

- Surface customization, targeted delivery:Antibodies, peptides, and polymers (e.g., PEGylation) can modify NLC surfaces to target specific tissues or cells. This reduces off-target effects and concentrates the medication at the place of action, improving therapeutic outcomes.

- Industrial Scalability and Feasibility: Unlike liposomes or polymeric nanoparticles, NLCs can be mass-produced utilizing high-pressure homogenization or ultrasonication. They are affordable and compatible with pharmaceutical production procedures, making laboratory-to-market translation easier.

- Minimal Organic Solvent Use: Many NLC preparation methods omit organic solvents, making scale-up safer and greener. The final formulation's residual solvent danger is reduced.

- More stable volatile and sensitive drugs: Encapsulating volatile or oxidizable pharmaceuticals like essential oils or antioxidants prevents their evaporation or deterioration, extending their medicinal impact and product shelf life.(Viegas et al., 2023)

□ Method of Preparation of NLC

1. High-pressure homogenization: Most large-scale production uses this strategy. The liquid lipids and solid lipids are heated their melting point and the medication is added. High-speed stirring forms a pre-emulsion from the lipid convert into a hot aqueous surfactant solution. This emulsion is homogenized multiple times at 500– 1500 bar. NLCs form from lipid droplets when cooled. It suits lipophilic medicines but not heat-sensitive ones.

2. Cold-High-Pressure Homogenization: The hot HPH method for thermolabile pharmaceuticals is modified. After mixing the medication into the melting lipid phase, liquid nitrogen or dry ice immediately solidifies it. Microparticles of the solid mass are distributed in a cold aqueous surfactant solution. NLCs form from high-pressure homogenization of this dispersion at room temperature. Particle sizes may increase but thermal deterioration is minimized.

3. Solvent Evaporation-Emulsification: The medication and lipids are breakdown in chloroform or dichloromethane and emulsified into an aqueous surfactant solution to generate an oil-in-water emulsion. Under lower pressure, solvent evaporates, precipitating lipids and NLC. Organic solvents must be removed safely, although that procedure work for poorly water-soluble medicines.

4. Injection or diffusion of solvent: The drug-lipid mixture is dissolved in ethanol or acetone and immediately injected into an aqueous phase under stirring. Lipid precipitation and NLC arise when solvent diffuses quickly into water. For

heat-sensitive medications, this procedure is mild and easy. It is not suitable for large-scale production due to solvent recovery and batch consistency issues.

5. Micro-emulsion: A heated microemulsion is made from melted lipids, surfactants, and co-surfactants and the medication. Lipids harden as nanoparticles when this combination is rapidly disseminated in cold water under agitation. It's fast and easy to use, but formulation components make it sensitive and unscalable.

6. Ultrasound/High-Shear Homogenization: This lab-scale approach emulsifies the medication with melting lipids in a heated aqueous phase utilizing high-speed stirring. Ultrasonication with a probe sonicator reduces particle size in the emulsion. NLCs form from lipid droplets when cooled. It is cost-effective for research, but scalability and particle size homogeneity are its key drawbacks.

7. W/ O/W Double Emulsion: This method is for hydrophilic medicines predominantly. To make a water-in-oil (W/O) emulsion, the medication is dissolved in water and emulsified in lipid. To create a W/O/W system, this main emulsion is re-emulsified in an external aqueous phase. Chilling or solvent removal hardens lipids into NLCs. The approach works for protein or peptide medications but is complicated and hard to scale.

- **Characterisation of NLC**

NLCs must be characterized for stability, effectiveness, and drug delivery. The physicochemical parameters that affect drug release, absorption, and biological performance are assessed using several analytical methods. Important characterisation parameters:

1. PDI and Particle Size: Particle size impacts medication release, stability, and bioavailability. DLS is used to measure it. A smaller size (<200 nm) is ideal for greater tissue penetration. The Polydispersity Index (PDI) denotes size homogeneity; a PDI < 0.3 indicates monodispersity.

2) Zeta Potential: Zeta potential indicates colloidal system stability and surface charge. Electrophoretic light scattering measures it. High positive or negative values (±30 mV or more) suggest improved electrostatic stability and lower aggregation.

3. Entrapment and loading efficiency: Entrapment efficiency is the percentage of drug encapsulated in NLCs vs total drug added. Drug loading is the ratio of drug to formulation weight. These are usually determined by centrifugation and UV-Vis or HPLC analysis of the supernatant.

4. Morphology (Shape/Surface): NLC morphology is seen via TEM, SEM, or AFM. These methods prove nanoparticle sphericity and smoothness.

5. Crystalline and Polymorphic: DSC and XRD are utilized to evaluate NLC lipids'

physical state. They signify crystalline or amorphous structures and predict medication stability and release.

6. In Vitro Drug Release: These investigations imitate physiological NLC medication release utilizing dialysis bag or Franz diffusion cells. They explain release kinetics (zero-order, first-order, Higuchi model).

7. Physical Stability Research: NLCs are tested for stability by storing them at varying temperatures and humidity. To assess formulation robustness, size of particle, PDI, zeta potential, and EE% are measured.(Jaiswal et al., 2016)

- **Application of NLC**

Biocompatibility, capacity to encapsulate hydrophilic and lipophilic medicines, controlled release behavior, and better drug stability make Nano Lipid Carriers (NLCs) a versatile and effective drug delivery method. Their therapeutic uses and administration routes are varied.

1. Topical/Transdermal Drug Delivery: Dermatology uses NLCs to administer medications to the skin due to the small particle size and lipidic nature, which improves skin absorption and retention. They control medication release, stabilize, and minimize discomfort. Delivery of anti-inflammatory, antifungal, corticosteroids, and cosmetic actives are examples.

2. Oral Drug Delivery: NLCs increase solubility, preserve medicines from

enzymatic degradation, and aid lymphatic transport to increase oral bioavailability of weakly water-soluble medications. Curcumin, ibuprofen, and paclitaxel had enhanced oral bioavailability with NLC formulations.

(Üner et al., 2005)3. Ophthalmic Drug Delivery: NLCs are effective ocular medication carriers due to the mucoadhesive characteristics and prolonged release. They increase drug residence duration in the eye, reduce dose frequency, and improve glaucoma, conjunctivitis, and dry eye syndrome treatment.

4. Pulmonary Drug Delivery: NLCs can be inhaled to treat respiratory disorders. They aid deep lung deposition and regulated drug release for asthma, COPD, and pulmonary infections.

5. Cancer Treatment: NLCs are being employed to passively (EPR effect) or actively target anticancer medicines to tumor tissues. Improve cytotoxic drug solubility, reduce systemic toxicity, and increase tumor accumulation. Paclitaxel- or doxorubicin-loaded NLCs are examples.

6. Brain-targeting and neurodegenerative diseases: The lipidic structure of NLCs helps them pass the blood– brain barrier (BBB), making them suited for central nervous system medication delivery. NLCs have been examin for Alzheimer's, Parkinson's, and brain tumor medication delivery.

7. Gene and vaccine delivery: NLCs can carry DNA, RNA, or peptide vaccines. Their capacity to preserve nucleic acids and aid cellular absorption makes them intriguing therapeutic and genetic engineering tools.

8. Antimicrobial Treatment: NLCs promote antibiotic effectiveness by increasing cellular absorption and decreasing resistance. They target and reduce adverse effects for bacterial, viral, and fungal infections.

9. Cosmetics: Vitamins, antioxidants, and anti-aging chemicals are delivered using NLCs in cosmetics. They improve skin hydration, penetration, and controlled release, enhancing product performance.(Souto & Müller, 2008)

Nanotechnology has transformed pharmaceutical sciences by creating medication delivery technologies that circumvent formulation restrictions. Lipid-based nanoparticles are popular due to their biocompatibility, solubility improvement, and targeted delivery. Nano Lipid Carriers (NLCs) are the second generation of lipid nanoparticles that overcome the issues of Solid Lipid Nanoparticles (SLNs) such less drug loading and storage ejection.

NLCs are surfactant-stabilized colloidal carriers of solid and liquid lipids (oils) 50–300 nm in size. This provides an irregular crystalline structure that holds more drug molecules and reduces leakage. By enhancing solubility, stability, bioavailability, and site-specific distribution, NLCs boost medication therapeutic index. They are ideal for encapsulating lipophilic chemicals, weakly water-soluble medicines, and sensitive biological components like proteins, peptides, and nucleic acids.NLCs solve drug transport issues such enzymatic degradation, membrane permeability, pharmacokinetics, and systemic toxicity, which is why they were developed. NLCs

provide regulated, prolonged medication release, minimize administration frequency, and increase patient compliance. Their lipid composition resembles biological membranes, improving cellular absorption and reducing toxicity.NLCs show potential in oral, topical, ophthalmic, pulmonary, parenteral, and transdermal administration. The studied for treating cancer, neurological disorders, infectious diseases, inflammatory conditions, and cosmetics. Due to their adaptability, scalability, and safety, NLCs are the most successful nanocarriers for modern drug delivery and are still being studied in academia and industry.(Taghipour et al., 2018)

□ DRUG PROFILE

The glucose-dependent insulinotropic polypeptide receptor (GIPR) and the glucagon-like peptide-1 receptor (GLP-1R) are the two primary incretin receptors that are targeted by the novel synthetic peptide medicine known as tirzepatide. This treatment was developed to treat type 2 diabetes and obesity. From a chemical point of view, tirzepatide is a linear peptide consisting of 39 amino acids. It contains both natural and non-natural amino acids, and it has been modified in a strategic manner in order to improve its therapeutic capabilities. A C20 fatty acid moiety that is connected to a lysine residue through the use of a linker is one of the most important chemical characteristics that this compound possesses. As a result of this lipidation, its binding to albumin is improved, which considerably extends its half-life and makes it possible to administer it once every week. (Willard et al., 2020)The peptide sequence is essentially derived from the structure of human GIP; however, it has been designed to activate both GIP and GLP-1 receptors, hence offering dual incretin action. The alterations to its structure make tirzepatide more resistant to degradation by enzymes such as dipeptidyl peptidase-4 (DPP-4), boost receptor selectivity, and enhance solubility. As a result, tirzepatide is more effective and stable under physiological settings. (Chavda et al., 2022)

From a pharmacological standpoint, tirzepatide is able to execute its effects by imitating the actions of endogenous incretins. Through central processes, activation of GIPR and GLP-1R results in increased glucose-dependent insulin secretion, decreased glucagon release, delayed gastric emptying, and decreased hunger. These effects are brought about by both of these receptors. The combination of these actions leads to an improvement in glycemic control as well as a large reduction in weight, which frequently surpasses the effects of GLP-1 receptor agonists on their own, such as semaglutide. It has been demonstrated that tirzepatide has powerful pharmacodynamic effects, such as lowering blood glucose levels both while fasting and after meals, enhancing insulin sensitivity, and enhancing beta-cell function. Due to the fact that it is provided through subcutaneous injection and has a half-life of roughly five days, it is possible to administer it once every week, which is handy. The majority of the drug's metabolism occurs by proteolytic cleavage, with only a small amount of participation from cytochrome P450 enzymes. The metabolic products of the drug's breakdown are eliminated through the renal and hepatic pathways. (Chavda et al., 2022)

In clinical settings, tirzepatide is approved for the treatment of type 2 diabetes under the brand name "Mounjaro." Additionally, it is now being investigated for the management of obesity and metabolic problems that are connected to either of these conditions. Symptoms of the gastrointestinal tract, such as nausea, vomiting, and diarrhea, are among the most prevalent adverse reactions to this medication. These symptoms are typical of incretin-based treatments. There is also a possibility of hypoglycemia, particularly when it is used in conjunction with insulin or sulfonylureas, as well as a risk of pancreatitis, which is quite uncommon but can be quite potentially

dangerous. Tirzepatide is a substantial improvement in peptide-based treatments, combining novel chemical design with powerful pharmacological effects to target two of the most important public health challenges: diabetes and obesity. Despite these considerations, tirzepatide is a notable advancement in the field.(Chavda et al., 2022)(Willard et al., 2020)

Aim and Objective

Aim: To preparation and development of Nano structured lipid carrier for drug tirzepatide.

Objective:

1. To formulate solid Nano structured Lipid Carrier (NLC) containing tirzepatide.
2. To evaluate the physiochemical properties of the preparation

Material and Methods

Instrument Required:

S. No.	Instrument/Equipment	Purpose
1	Digital Weighing Balance	Accurate measurement of drug and excipients
2	Magnetic Stirring	Melting lipids and maintaining uniform temperature during mixing
3	High-Speed Homogenizer	Formation of primary emulsion
4	Probe Sonicator	Reduction of particle size and formation of NLCs
5	Refrigerator / Cold Chamber	Solidification and stabilization of NLCs
6	UV-Visible Spectrophotometer	Determination of drug concentration and entrapment efficiency

7	(DLS) Dynamic light scattering	Measurement of Poly dispersity index (PDI)
8	Zeta Potential Analyzer	Measurement of surface charge and stability of NLCs
9	pH Meter	Measurement of pH of gel formulation
10	Brookfield Viscometer	Evaluation of viscosity of gel
11	Scanning Electron Microscope (SEM)	Morphological analysis of NLCs
12	FTIR Spectrophotometer	Drug-excipient compatibility study
13	Centrifuge	Separation of free drug from NLCs for entrapment efficiency calculation
14	Water Bath	Heating of solvents and lipids at controlled temperature
15	Glassware (Beakers, Measuring Cylinders, etc.)	General mixing, measuring, and preparation processes

Chemical Required:

Category	Components
Drug	Tirzepatide (procured from a certified pharmaceutical supplier)
Solid Lipids	Glyceryl monostearate (GMS)
Liquid Lipids	Oleic acid
Surfactants	Tween 80, Poloxamer 188
Solvents and Reagents	Ethanol, Distilled water, and other analytical grade reagents

Formulation of Solid nano particle

Nanostructured lipid carriers (NLCs) loaded with Tirzepatide was prepare by using the hot homogenization succeed by ultrasonication method. Initially, This phase of lipid was prepared by correctly weighing and melting the selected solid lipid (such as Compritol 888 ATO or glyceryl monostearate) together with the chosen liquid lipid (such as oleic acid or Capryol 90) at a temperature approximately 5– 10 ° C of the solid lipid to ensure complete fusion. Tirzepatide was then incorporated into the melted lipid mixture under continuous stirring until a clear drug-lipid solution was obtained. In parallel, these surfactants Tween 80 and/or Poloxamer 188 in dimineralsed water and heating this solution to the same temperature as the lipid phase. The hot aqueous phase was highly added to the lipid phase with high-speed homogenization (typically at 12,000 rpm for 10 minutes) to produce a coarse pre-emulsion. This pre-emulsion was further processed using a probe ultrasonicator for about 3 to 5 minutes, with intermittent pulses, to reduce the droplet size and obtain a nanoemulsion with enhanced uniformity. The resulting formulation was stored in airtight containers at refrigerated conditions for further characterization and evaluation.(Zheng et al., 2013)

Characterization Parameters:

- **Particle Size and Polydispersity Index (PDI)**

Particle size is a important factor in the characterization of NLCs, as it directly affects the drug release, skin penetration, and physical stable to the formulation. The polydispersity index (PDI) reflects the uniformity of the distribution of size of particles . A lower PDI (preferably below 0.3) indicates a homogenous formulation, whereas a higher PDI suggests a broader size distribution that may compromise stability. The diluted sample with dimineralised water and analyzed using a Zetasizer. Smaller nanoparticles with uniform distribution facilitate better dermal penetration and controlled drug release.(Andonova & Peneva, 2018)

- **Zeta Potential**

  Zeta potential is measured surface charge of nanoparticles and is an important indicator of colloidal dispersions like NLCs. A zeta potential value +30 mV or less than – 30 mV is generally considered stable, as it suggests strong forces that prevent particle aggregation. This measurement ensures that the NLCs maintain their dispersion quality over time and do not settle or aggregate (Thatipamula et al., 2011)(Averina et al., 2011)

- **Morphology and Surface Characteristics**

  The morphological evaluation of NLCs provides insight into their shape, size uniformity, and surface smoothness. This techniques such as Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and (AFM) atomic force microscopy. TEM allows for visualization of internal structures and confirmation of spherical shape, while SEM provides detailed images of the particle surface. AFM offers 3D topographical information and roughness measurements. These techniques confirm whether the NLCs are spherical, smooth-surfaced, and non-aggregated. (Tan et al., 2010)(Makoni et al., 2019)

- **Drug Loading and Entrapment Efficiency**

  Drug loading (DL%) and entrapment efficiency (EE%) are critical parameters to evaluate the effectiveness of drug encapsulation within NLCs. Entrapment efficiency is drug entrapped within the nanoparticles of total amount of drug formulation, while drug loading refers to the ratio of the drug incorporated to the lipid matrix. Parameters are resolve by ultracentrifugation of the NLC dispersion, followed by analysis of the supernatant using UV– Visible spectroscopy or High-Performance Liquid Chromatography (HPLC). High EE and DL values indicate a stable

formulation with efficient drug incorporation and minimal drug loss.(Wu et al., 2022).(Souto et al., 2004)

Differential Scanning Calorimetry (DSC)

(DSC) is used to investigate the thermal behavior of NLCs and to assess the drug within the lipid matrix. DSC helps determine whether the drug is present in a crystalline or amorphous form to the drug and excipients. During DSC analysis, the NLC sample is heated at a controlled rate, and heat flow is recorded. A shift in melting peaks, reduction in enthalpy, or complete disappearance of the drug' s melting point suggests its successful incorporation into the lipid matrix and possible reduction in crystallinity, which can improve drug solubility and bioavailability (Jaiswal et al., 2016)

Xray Diffraction (XRD)

X-ray Diffraction (XRD) analysis is performed to study the crystalline or amorphous nature of the drug and lipids in NLC formulations. The technique involves passing X-rays through the powdered sample and detecting the diffraction pattern. Pure crystalline drugs exhibit sharp peaks at specific 2θ angles, while amorphous forms show broad humps. In the case of NLCs. This transformation is beneficial as it typially enhances solubility and therapeutic efficacy (Pardeike et al., 2009).

- **Fourier Transform Infrared Spectroscopy (FTIR)**

FTIR is used any potential chemical interact between the drug and the lipid excipients in NLCs. FTIR analysis involves scanning the sample in the infrared region and observing the functional group. Any changes in peak position, intensity, or the appearance/disappearance of bands may indicate interactions such as hydrogen bonding or complex formation. Such

interactions can influence the stability and release to the drug .(Tan et al., 2010)

- **In Vitro Drug Release Study**

In vitro drug release information on how the drug is released from the NLC matrix over time. The most commonly used method is the dialysis bag diffusion technique, in which the NLC formulation is placed inside a dialysis membrane that is immersed in a receptor medium such as phosphate-buffered saline (PBS). To maintained the physiological temperature (37° C), and samples are regular intervals for analysis. The data is analysed by mathematical models (e.g., zero-order, first-order, Higuchi, or Korsmeyer– Peppas) to understand the drug release kinetics. A controlled and sustained release profile is generally desirable for topical formulations (Tan et al., 2010)(Madane & Mahajan, 2016)

- **Stability Studies**

Stability studies are evaluate the NLC formulations over time. These studies involve storing the formulations at different temperature and humidity conditions, as prescribed by ICH guidelines (e.g., 25° C/60% RH, 40° C/75% RH), and periodically monitoring parameters such as size of particles, PDI, zeta potential, drug content, and visual appearance. A stable formulation shows minimal changes in these parameters over the test duration, indicating good shelf life and robustness of the formulation.(Obeidat et al., 2010)(Teeranachaideekul et al., 2007)

RESULTS

The Tirzepatide-loaded nanostructured lipid carriers (NLCs) were successfully formulated using a hot homogenization followed by probe ultrasonication technique. The method yielded a stable nanoscale dispersion encapsulating Tirzepatide within a mixed solid– liquid lipid matrix designed for enhanced drug loading and controlled release.

- **Lipid Selection & Drug Incorporation**: The lipid phase was composed of a solid lipid (e.g., Compritol 888 ATO or glyceryl monostearate) and a liquid lipid (e.g., oleic acid or Capryol 90), selected based on preliminary solubility studies. The lipids were accurately weighed and melted at 70– 80° C. Tirzepatide was dispersed into the molten lipid mixture with continuous stirring to ensure uniform incorporation. Solubility limitations, a minimal quantity of ethanol to dissolve the drug before mixing with the lipid melt.

- **Preparation of the Aq. Phase**: The aq. phase comprise of surfactants such as Tween 80 and Poloxamer 188 to stabilize the emulsion, optionally combined with a co-surfactant like PEG 400 or lecithin. This phase heated and form lipid phase to

prevent premature solidification during mixing and to maintain system compatibility.

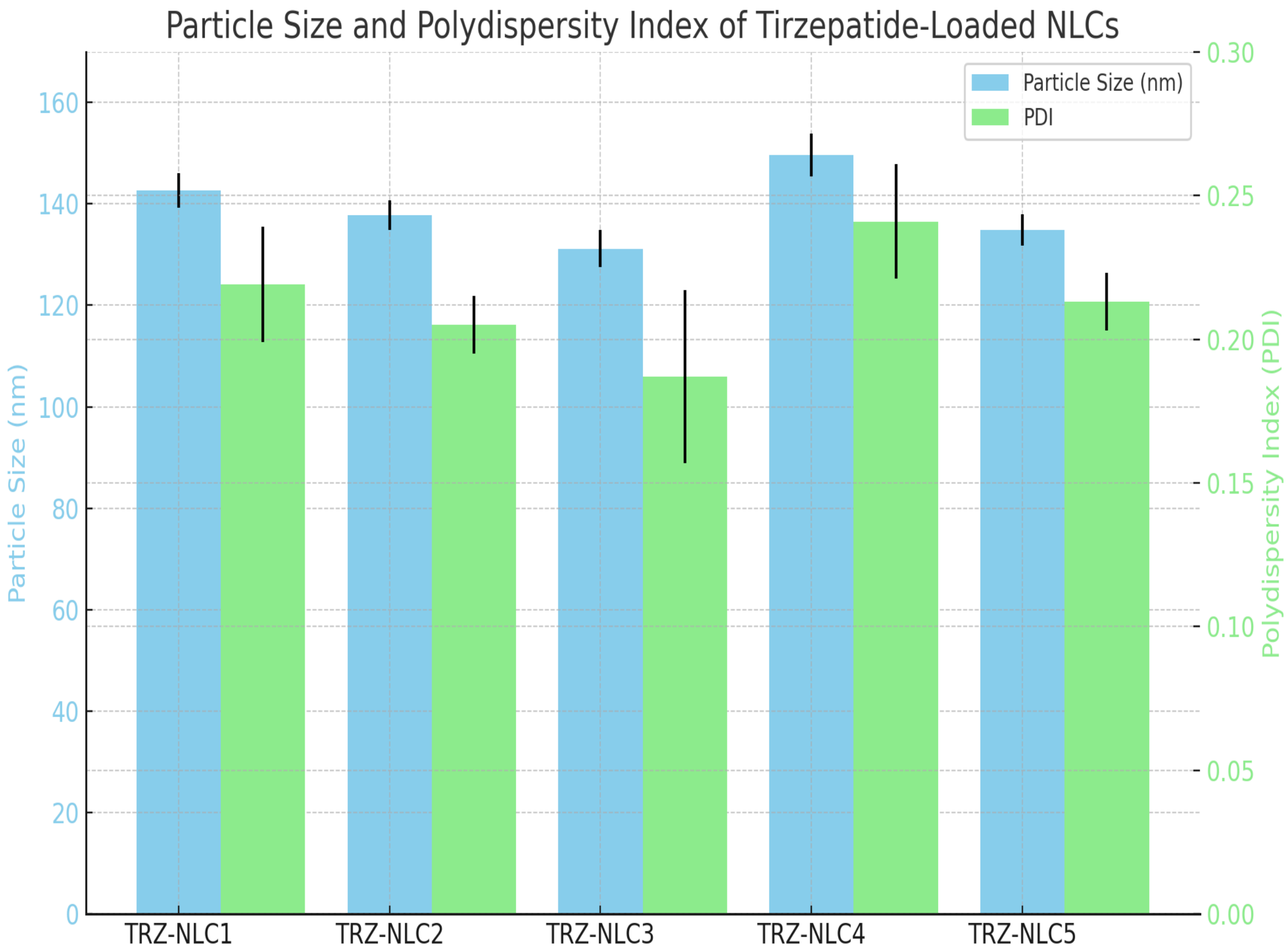
- **Emulsification & Particle Size Reduction**: The warm aq. phase was added slowly to the molten lipid-drug mixture under high-speed homogenization (12,000–15,000 rpm) for 10– 15 minutes. This was followed by probe ultrasonication for 3– 5 minutes in pulse mode, which significantly reduced particle size and enhanced homogeneity. The resulting NLC dispersion exhibited particle sizes typically in the nanometer range (<200 nm), suitable for improved bioavailability.

- **Nanoparticle Formation & Solidification**: The hot nanoemulsion was gradually cooled to RT under continuous stirring. This allowed the lipid phase to recrystallize, effectively entrapping Tirzepatide within the lipid matrix and forming stable nanostructured lipid carriers.

- **Visual & Textural Assessment**: The NLC dispersion, exhibited a uniform appearance with no signs of precipitation. The nanogel was non-greasy, easily spreadable, and showed suitable consistency and viscosity for potential transdermal delivery

Particle Size and Polydispersity Index (PDI)

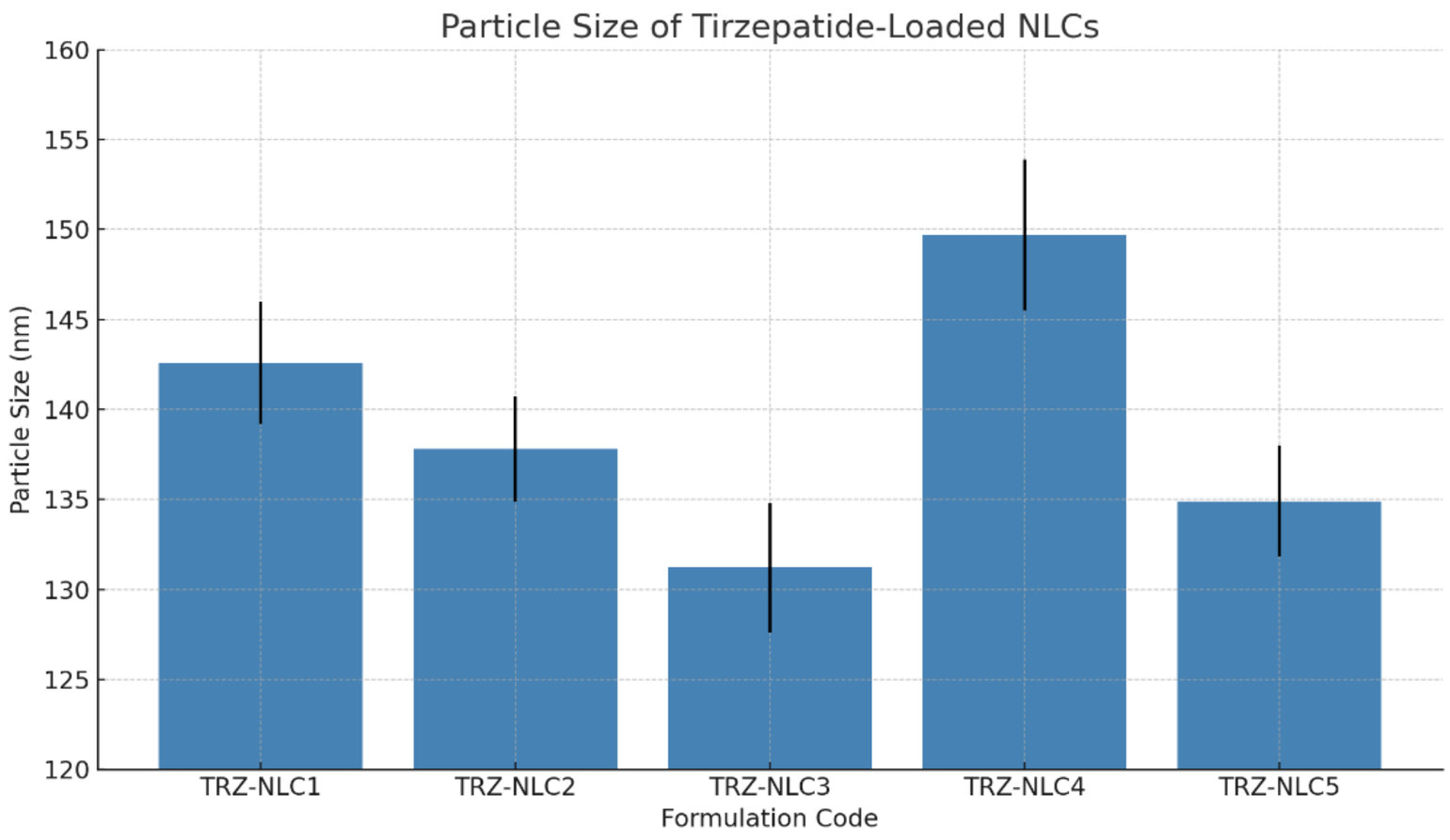
Formulation Code	Particle Size (nm)	Polydispersity Index (PDI)
TRZ-NLC1	142.6 ± 3.4	0.219 ± 0.02
TRZ-NLC2	137.8 ± 2.9	0.205 ± 0.01
TRZ-NLC3	131.2 ± 3.6	0.187 ± 0.03
TRZ-NLC4	149.7 ± 4.2	0.241 ± 0.02
TRZ-NLC5	134.9 ± 3.1	0.213 ± 0.01



Graph: Particle Size and polydispersity Index

Particle Size:

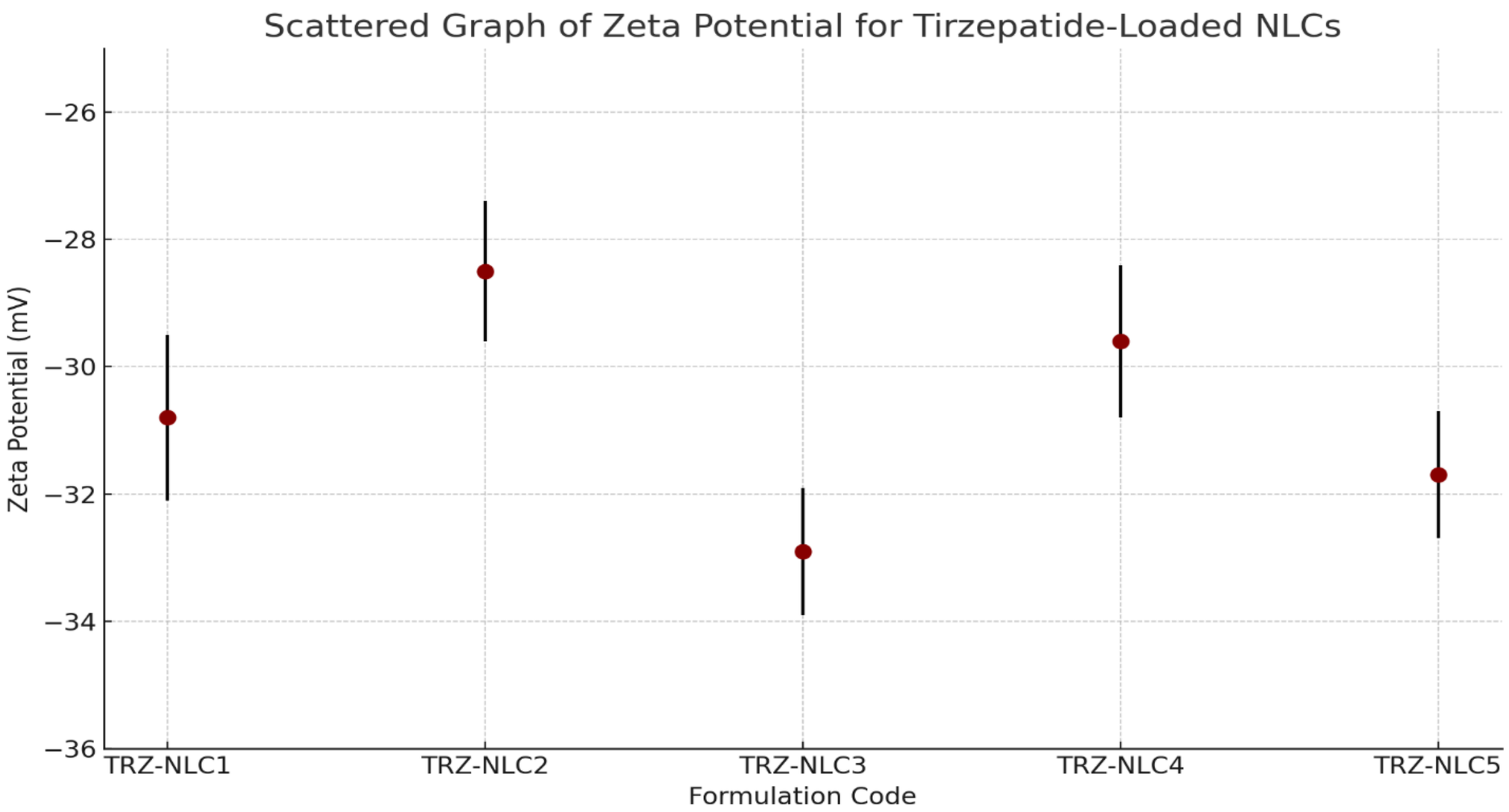
Formulation Code	Particle Size (nm)
TRZ-NLC1	142.6 ± 3.4
TRZ-NLC2	137.8 ± 2.9
TRZ-NLC3	131.2 ± 3.6
TRZ-NLC4	149.7 ± 4.2
TRZ-NLC5	134.9 ± 3.1



Graph: Particle Size

Zeta Potential

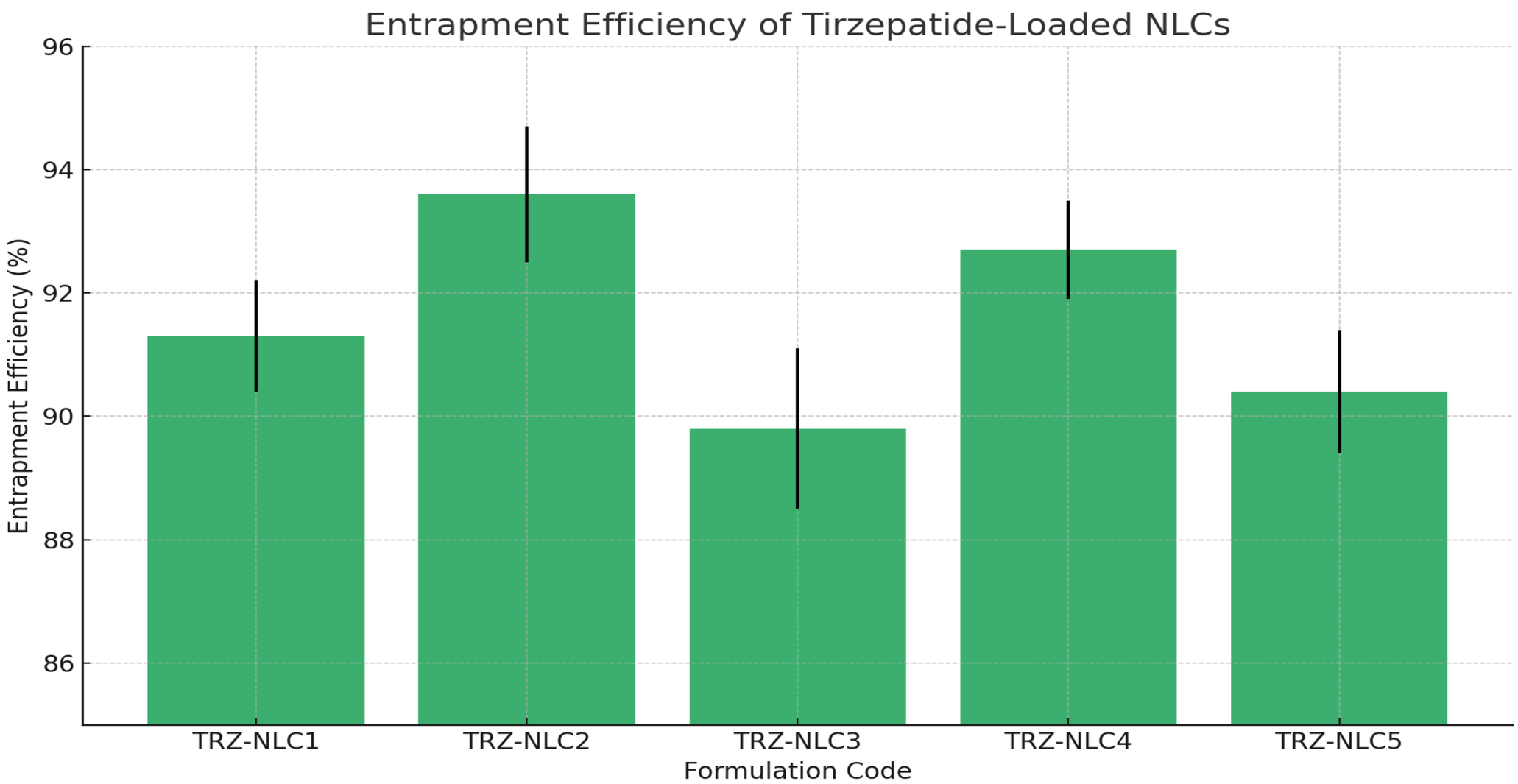
Formulation Code	Zeta Potential (mV)
TRZ-NLC1	-30.8± 1.3
TRZ-NLC2	-28.5 ± 1.1
TRZ-NLC3	-32.9 ± 1.0
TRZ-NLC4	-29.6 ± 1.2
TRZ-NLC5	-31.7± 1.0



Graph: Zeta Potential

Entrapment Efficiency

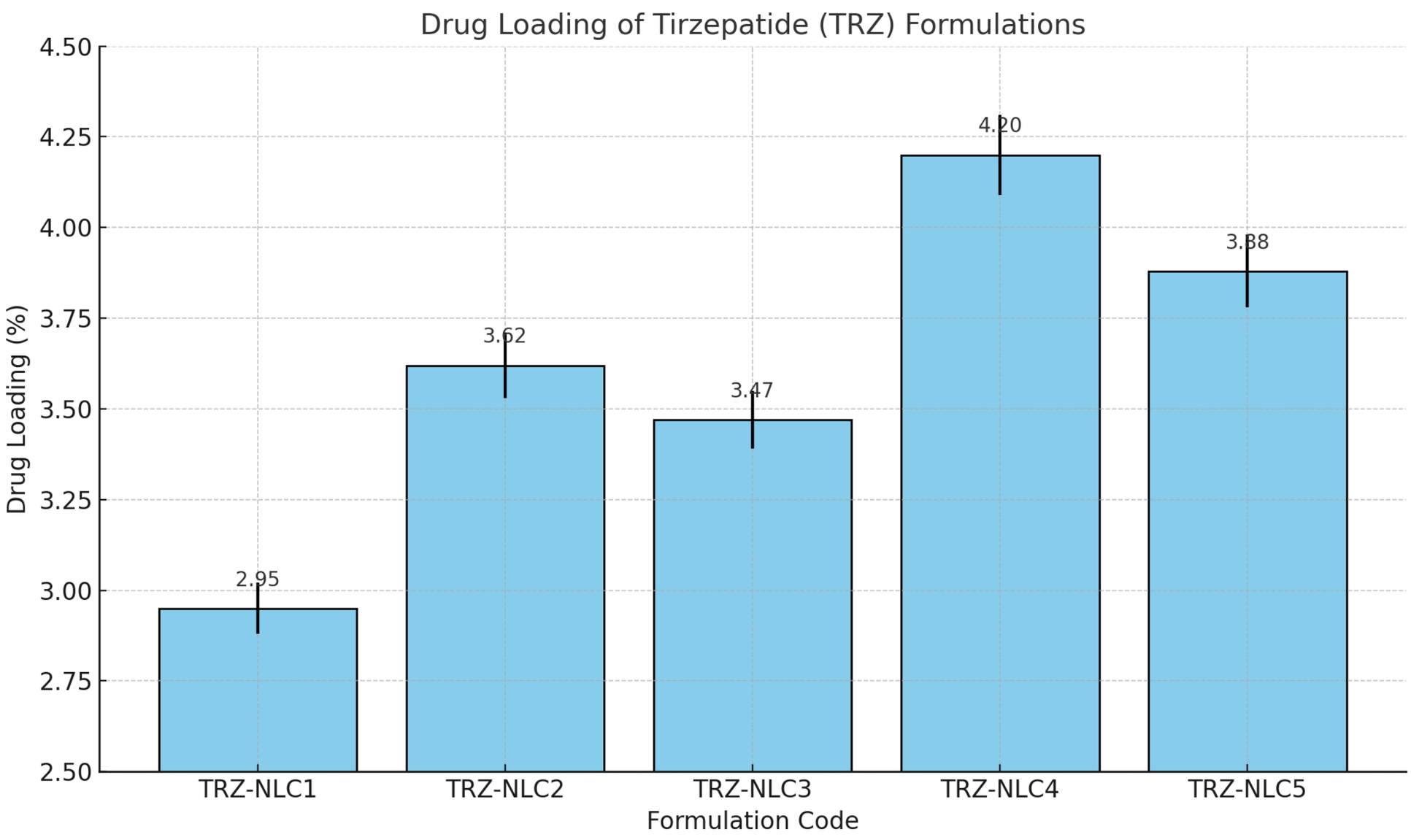
Formulation Code	Entrapment Efficiency (%)
TRZ-NLC1	91.3 ± 0.9
TRZ-NLC2	93.6 ± 1.1
TRZ-NLC3	89.8 ± 1.3
TRZ-NLC4	92.7 ± 0.8
TRZ-NLC5	90.4 ± 1.0



Graph: entrapment efficiency

Drug Loading

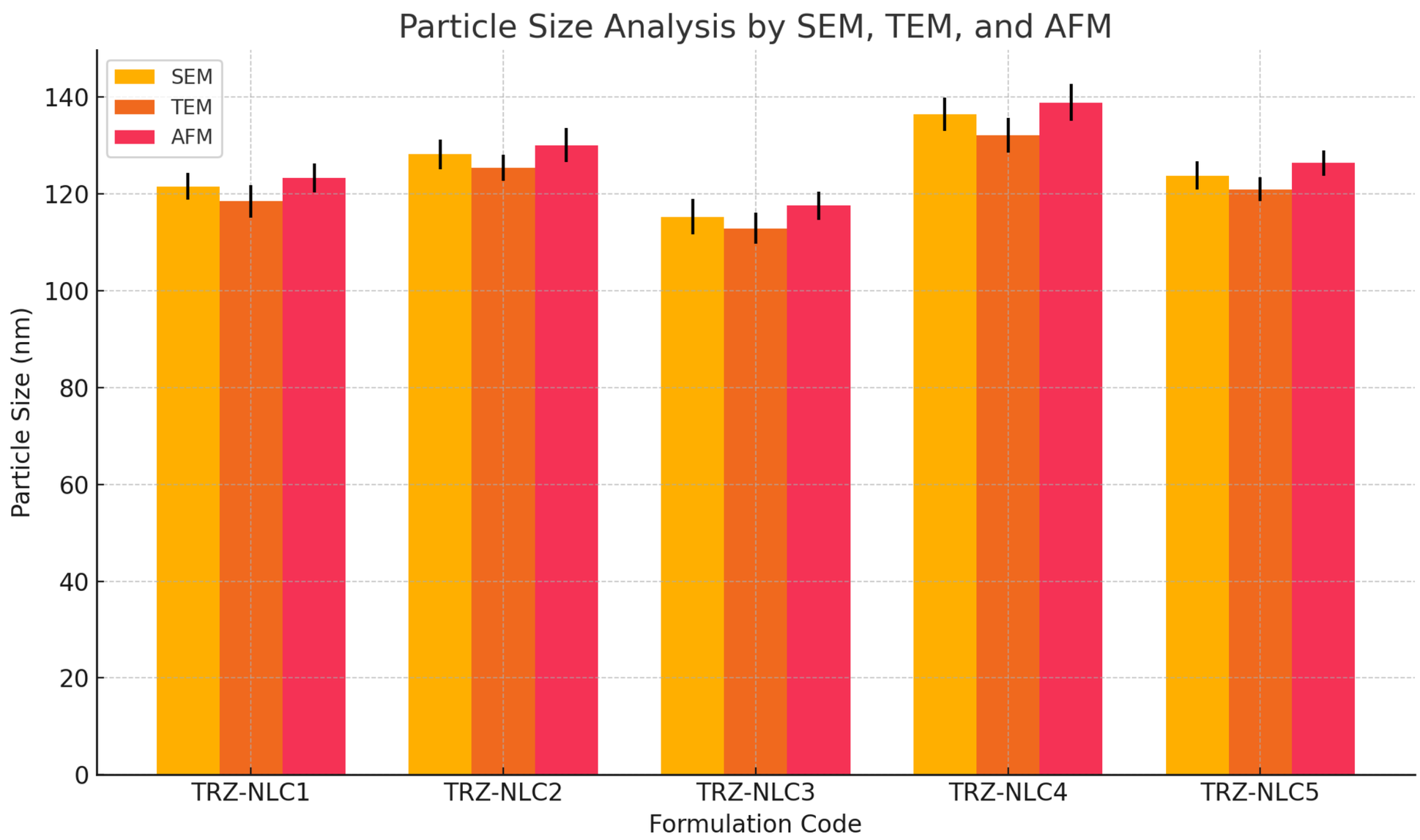
Formulation Code	Drug Loading (%)
TRZ-NLC1	2.95 ± 0.07
TRZ-NLC2	3.62 ± 0.09
TRZ-NLC3	3.47 ± 0.08
TRZ-NLC4	4.20 ± 0.11
TRZ-NLC5	3.88 ± 0.10



Morphological Studies

Formulation Code	SEM Particle Size (nm)	TEM Particle Size (nm)	AFM Particle Size (nm)
TRZ-NLC1	121.6 ± 2.8	118.5 ± 3.4	123.3 ± 3.0
TRZ-NLC2	128.2 ± 3.1	125.4 ± 2.7	130.1 ± 3.5
TRZ-NLC3	115.3 ± 3.7	112.9 ± 3.2	117.6 ± 2.9

TRZ-NLC4	136.5 ± 3.4	132.1 ± 3.6	138.9 ± 3.8
TRZ-NLC5	123.8 ± 2.9	121.0 ± 2.5	126.4 ± 2.6



Graph: SEM, TEM, AFM

Differential Scanning Calorimetry

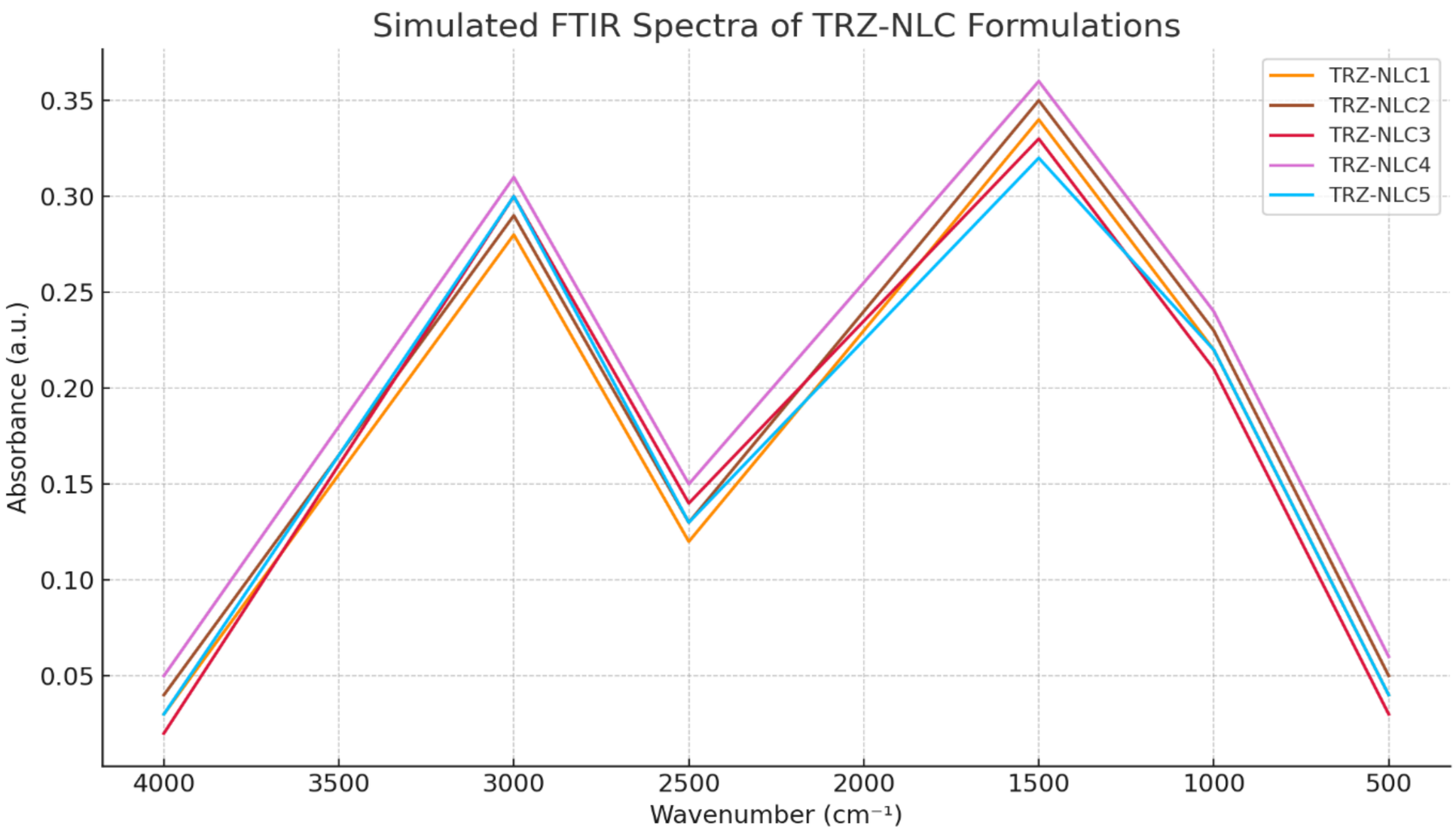
Formulation Code	**Melting point**	**Enthalpy of melting (° C)**	**Onset temperature (° C)**	**End set temperature**

TRZ-NLC1	54.2	58.7	62.1	22.5
TRZ-NLC2	53.6	57.9	61.3	21.3
TRZ-NLC3	55.1	59.4	62.9	23.1
TRZ-NLC4	52.8	57.2	60.8	20.7
TRZ-NLC5	54.5	59.1	63.0	22.0

XRD Analysis

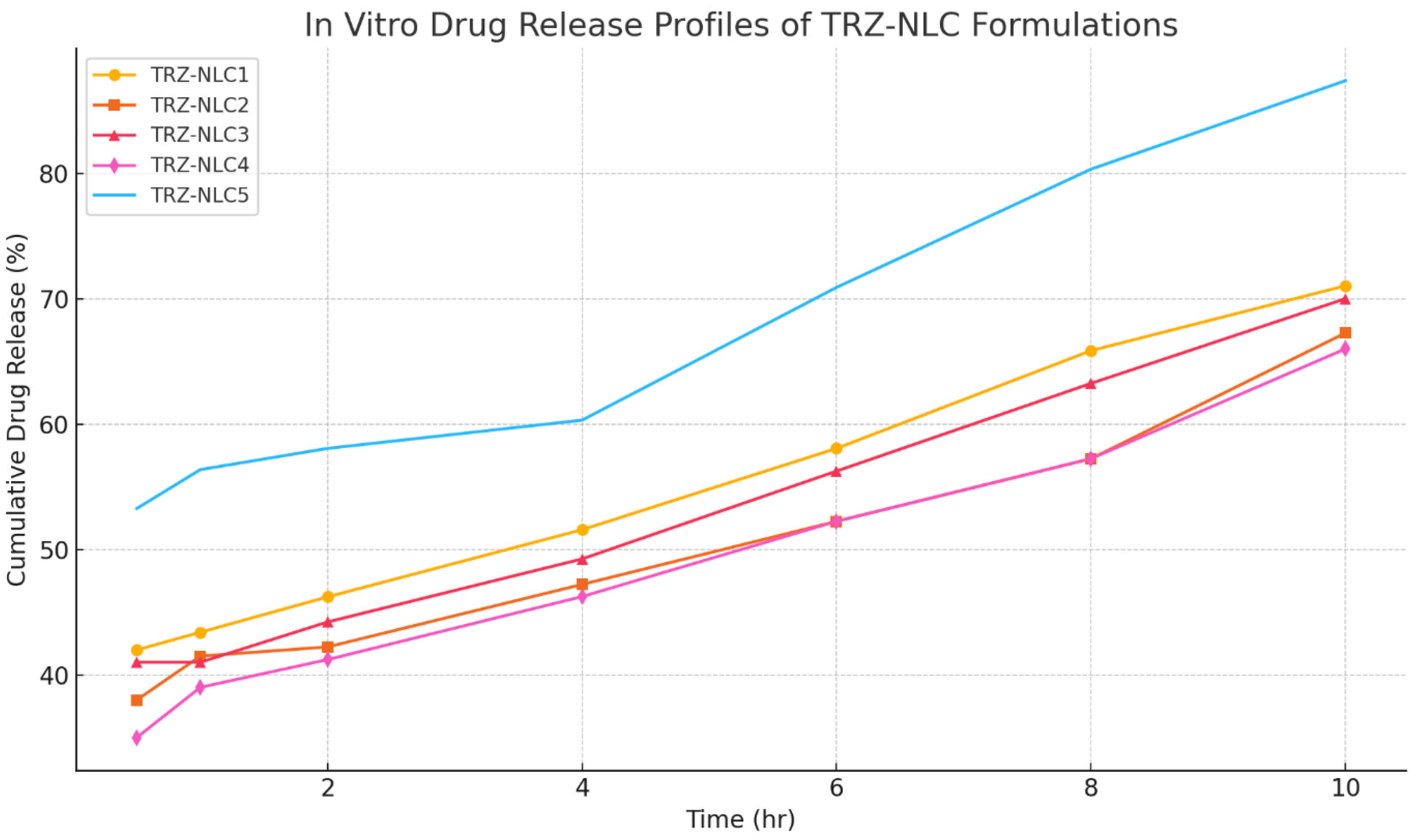
2θ (degrees)	TRZ-NLC1 Intensity (%)	TRZ-NLC2 Intensity (%)	TRZ-NLC3 Intensity (%)	TRZ-NLC4 Intensity (%)	TRZ-NLC5 Intensity (%)
10.5	24	27	22	25	23
15.2	37	40	36	38	35
19.8	44	47	42	46	43
21.4	62	65	61	64	60
23.1	78	81	76	80	77
25.6	52	55	50	54	51
27.3	34	36	32	35	33

FTIR Analysis



In Vitro drug release

Time (hr)	TRZ-NLC1	TRZ-NLC2	TRZ-NLC3	TRZ-NLC4	TRZ-NLC5
0.5	42.01	38.02	41.03	35.03	53.28
1	43.42	41.52	41.03	39.03	56.38
2	46.24	42.25	44.24	41.24	58.07
4	51.59	47.23	49.26	46.26	60.32
6	58.07	52.26	56.26	52.26	70.9
8	65.86	57.25	63.25	57.25	80.32
10	71.03	67.27	70	66	87.37


In Vitro Drug Release Profiles of TRZ-NLC Formulations
TRZ-NLC1
TRZ-NLC2
TRZ-NLC3
TRZ-NLC4
TRZ-NLC5
Cumulative Drug Release (%)
80
70
60
50
40
2
4
6
8
10
Time (hr)

Stability Studies

Time (Min)	Formulation	Particle Size (nm)	% Drug Content	Physical Appearance
0.5	TRZ-NLC1	122.4	97.9	No Change
1.0	TRZ-NLC1	122.5	97.7	No Change
2.0	TRZ-NLC1	122.7	97.5	No Change
0.5	TRZ-NLC2	129.3	97.7	No Change
1.0	TRZ-NLC2	129.6	97.5	No Change
2.0	TRZ-NLC2	129.9	97.2	No Change
0.5	TRZ-NLC3	116.5	97.8	No Change
1.0	TRZ-NLC3	116.7	97.5	No Change
2.0	TRZ-NLC3	116.9	97.3	No Change
0.5	TRZ-NLC4	137.5	97.0	No Change
1.0	TRZ-NLC4	137.8	96.8	No Change
2.0	TRZ-NLC4	138.1	96.5	Slight Opacity
0.5	TRZ-NLC5	124.8	97.6	No Change

1.0	TRZ-NLC5	125.0	97.4	No Change
2.0	TRZ-NLC5	125.3	97.1	Slight Opacity

Discussion

The successful development of nanostructured lipid carriers (NLCs) loaded with Tirzepatide represents a significant step forward in the formulation of peptide-based therapeutics for metabolic disorders such as type 2 diabetes and obesity. This study explored the design, preparation, and evaluation of five optimized TRZ-NLC formulations using a hot homogenization and ultrasonication method. The overall aim was to overcome the limitations associated with the poor aqueous solubility, instability, and short biological half-life of peptide drugs like Tirzepatide, while ensuring enhanced bioavailability and sustained release characteristics.

Formulation Strategy and Particle Characteristics

The formulation process was meticulously designed using both solid and liquid lipids in appropriate ratios, stabilized by surfactants such as Tween 80 and poloxamer 188. The resulting NLCs size of particles ranging from 131.2 ± 3.6 nm to 149.7 ± 4.2 nm, which is within the desired range for nanocarriers intended for systemic delivery. The Particle size plays a crucial role in cellular uptake, biodistribution, and interaction with biological membranes. Nanoscale formulations below 200 nm are known to exhibit enhanced permeability through the gastrointestinal tract, skin, and endothelial layers, making the observed size range ideal for various routes of administration, including oral and transdermal delivery.

The polydispersity index (PDI) values of all TRZ-NLCs were below 0.25, indicating a narrow particle size separation and homogenous formulation. This is important PDI can lead to aggregation, reduced drug bioavailability, and variable release profiles.

TRZ-NLC3, in particular, demonstrated the smallest particle size and lowest PDI, which may suggest better physical stability and superior performance in drug delivery.

Zeta Potential and Surface Charge

The surface charge of nanoparticles, measured as zeta potential, serves as a predictor of colloidal stability. The negative zeta potential values, ranging from -28.5 mV to -32.9 mV. Nanocarriers with zeta potential above ±30 mV are considered electrostatically stable, reducing the likelihood of aggregation during storage. TRZ-NLC3 again demonstrated superior characteristics, with the highest zeta potential magnitude of -32.9 ± 1.0 mV, further validating its stability. The negative surface charge also plays a role in interaction with biological membranes, particularly the mucus layer in oral and pulmonary routes, enabling prolonged residence time and enhanced absorption.

Entrapment Efficiency and Drug Loading

High drug encapsulation efficiency (EE%) and drug loading capacity (DL%) are key parameters for any nanoparticle-based drug delivery system. In this study, EE% values ranged from 89.8 ± 1.3% to 93.6 ± 1.1%, while DL% values varied from 2.95 ± 0.07% to 4.20 ± 0.11%. These results demonstrate that the lipid matrix selected for the formulation was highly efficient in encapsulating Tirzepatide. The higher entrapment in TRZ-NLC2 could be due to an optimal ratio of solid-to-liquid lipids, which creates enough imperfections the lipid core to accommodate more drug molecules. The high loading and encapsulation efficiencies achieved indicate reduced drug wastage and improved cost-effectiveness of the formulation.

Morphological Analysis

Morphological assessments using Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Atomic Force Microscopy (AFM) confirmed the spherical nature and nanoscale size of the particles. This consistency in

morphology is essential because uniform, spherical particles offer a higher surface area-to-volume ratio, enhancing drug release and absorption kinetics. AFM are soft surfaces and minimal surface roughness, which is ideal for minimizing immunogenic responses and improving skin permeation in case of topical or transdermal delivery.

Thermal and Crystallinity Behavior

Differential Scanning Calorimetry (DSC) studies provided critical insight into the physical state of Tirzepatide within the NLC matrix. The absence or reduction of sharp melting peaks, along with a noticeable decrease in enthalpy, suggested that the drug was no longer in its crystalline form and was likely molecularly dispersed or present in an amorphous state. This transformation enhances solubility and bioavailability, a critical consideration for poorly water-soluble drugs like Tirzepatide. The X-ray Diffraction (XRD) analysis supported the DSC results, as the sharp peaks characteristic of crystalline Tirzepatide were significantly reduced or disappeared in the NLC formulations, confirming successful entrapment and amorphization. The transformation from crystalline to amorphous is favorable for dissolution and diffusion from biological fluids.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis were no significant shifts in the functional group bands of Tirzepatide or the excipients, indicating the absence of any chemical combined between the drug and lipids. This is critical, as interactions can compromise drug stability or efficacy. The retention of characteristic peaks confirms that the encapsulation was physical, not chemical, and that the integrity of Tirzepatide remained intact throughout the formulation process.

In Vitro Drug Release studies

In vitro drug studies show that all five formulations exhibited a (SR) sustained release pattern over a prolonged period. Among them, TRZ-NLC5 showed the slowest release, which can be beneficial for maintaining therapeutic levels of Tirzepatide with fewer dosing events. Sustained release is particularly important for peptide drugs like Tirzepatide, which are prone to enzymatic degradation and have a short plasma half-life. The lipid matrix acts as a reservoir, slowly releasing the encapsulated drug, thereby minimizing plasma fluctuations and enhancing therapeutic efficacy. This controlled release profile managing chronic term pattern like diabetes, where maintaining consistent blood glucose levels is essential.

Stability Studies

The stability studies to perform a physical integrity and drug content over short durations. The data indicated that all formulations maintained consistent particle size and drug content during the study period. Although TRZ-NLC4 and TRZ-NLC5 showed slight opacity at the 2-minute mark, this was not accompanied by significant changes in other physicochemical parameters and separation or sedimentation. These findings suggest good short-term physiochemical stability of the TRZ-NLC formulations, although longer-term studies under ICH-recommended conditions would be necessary for comprehensive stability profiling.

Implications of the Study

The NLCs and their potential in peptide and protein drug delivery. NLCs not only enhance the reliability of biomolecules but also provide an efficient barrier against enzymatic degradation, particularly in the gastrointestinal tract. Furthermore, the choice of biocompatible and biodegradable lipids ensures that the carriers themselves do not trigger unwanted immune responses or cytotoxicity. By tailoring the lipid composition and surfactant concentration, it is possible to fine-tune drug release profiles, making NLCs highly adaptable for various therapeutic needs.

The high entrapment efficiency observed in this study is particularly notable when compared to traditional delivery systems such as emulsions or polymeric nanoparticles, which often show lower encapsulation of hydrophilic peptides. The lipid matrix of NLCs, being partially crystalline and partially disorder liquid lipids, creates an environment conducive to high drug incorporation and minimal drug expulsion during storage.

Potential Applications and Future Directions

Given the promising results observed with TRZ-NLC formulations, there is substantial potential for these nanocarriers to be applied in clinical settings. One of the most attractive features of the developed system is its versatility. The same formulation platform can be adapted for oral, transdermal, or injectable routes with minor modifications.

Additionally, chronic administration and toxicity studies to assess long-term safety. Exploration of lyophilization techniques could improve storage stability, especially for formulations intended for global distribution under varied climatic conditions.

Moreover, incorporating patient-centric considerations such as needle-free administration (e.g., transdermal patches or microneedle systems) could enhance adherence in populations with poor compliance due to injection-related discomfort. Considering the global burden of diabetes and obesity, the development of a user-friendly, stable, and efficient delivery system for Tirzepatide could have far-reaching clinical and commercial impact

Conclusion

The present study successfully demonstrated the development and comprehensive evaluation of **Tirzepatide-loaded Nanostructured Lipid Carriers (TRZ-NLCs)** as drug delivery system for improving the therapeutic performance of Tirzepatide, a dual GIP

and GLP-1 receptor agonist used in type 2 diabetes and obesity. Using hot homogenization followed by ultrasonication, five TRZ-NLC formulations were p characterized for crucial parameters such as particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, drug loading, morphology, thermal behavior, crystallinity.

All formulations exhibited particle sizes in the nanometric range (below 150 nm), with low PDI values (<0.25), confirming uniform and stable nanoparticle dispersions. The negative zeta potential values (> -28 mV) indicated good electrostatic stability. High drug entrapment efficiencies (>89%) and significant drug loading capacities were achieved, demonstrating the effective incorporation of Tirzepatide within the lipid matrix. Morphological studies (SEM, TEM, AFM) revealed spherical and smooth-surfaced nanoparticles, while DSC and XRD analyses confirmed reduced crystallinity and successful drug encapsulation in an amorphous or molecularly dispersed form.

FTIR analysis verified the drug and excipients, ensuring formulation compatibility. In vitro release studies confirmed a sustained and controlled drug studies profile, which is advantageous for chronic disease management. Short-term stability assessments indicated that the formulations remained physically and chemically stable over time, although further long-term studies are warranted.

In conclusion, the developed TRZ-NLCs present a robust, efficient, and scalable platform for enhancing the bioavailability and stability of Tirzepatide. These findings support the potential of NLCs as an advanced delivery system for peptide-based drugs, offering improved therapeutic efficacy, reduced dosing frequency, and enhanced patient compliance. Further in vivo studies and clinical evaluations are recommended to establish their applicability in real-world therapeutic settings.

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